1 **Life in the fast lane: temperature, density and host species impact survival and growth of**
2 **the fish ectoparasite *Argulus foliaceus***
3

4 *Rhi Hunt[a]* & Jo Cable[a]*
5   [a.] School of Biosciences, Cardiff University, Cardiff CF10 3AX, UK.
6   * Corresponding Author: HuntRC1@Cardiff.ac.uk

7

8 **Abstract**

9 With expanding human populations, the food sector has faced constant pressure to
10 sustainably expand and meet global production demands. In aquaculture this frequently
11 manifests in an animal welfare crisis, with fish increasingly farmed under high production,
12 high stress conditions. These intense environments can result in fish stocks having a high
13 susceptibility to infection, with parasites and associated disease one of the main factors
14 limiting industry growth. Prediction of infection dynamics is key to preventative treatment
15 and mitigation. Considering the climatic and technology driven changes facing aquaculture,
16 an understanding of how parasites react across a spectrum of conditions is required. Here we
17 assessed the impact of temperature, infection density and host species on the life history
18 traits of *Argulus foliaceus,* a common palearctic fish louse, representative of a parasite group
19 problematic in freshwater aquaculture and fisheries worldwide. Temperature significantly
20 affected development, growth and survival; parasites hatched and developed faster at higher
21 temperatures, but also experienced shorter lifespans when maintained off the host. At high
22 temperatures, these parasites will likely experience a short generation time as their life
23 history traits are completed more rapidly. *A. foliaceus* additionally grew faster on natural
24 hosts and at lower infection densities. Ultimately such results contribute to prediction of
25 population dynamics, aiding development of effective control to improve animal welfare and
26 reduce industry loss.

27

28 **Keywords**
29 Life history; Aquaculture; Climate change; Fish pathogen; Infectious disease
30

31 **1. Introduction**
32 Aquaculture has global economic benefits, providing food security and supplying stock for
33 sport fishing and the ornamental pet trade (FAO 2018). As fisheries intensify to meet global
34 demands, animals are subject to an increasing number of stressors (Wood 2001; Lewin *et al.*
35 2006; Wedemeyer *et al.* 1997). Such conditions facilitate and amplify parasite transmission
36 and disease outbreaks, with infections arguably the most important factor limiting
37 sustainable industry expansion (Granada *et al.* 2016). Management of parasites and disease
38 in fish is grossly lacking compared to mammalian species (Burka *et al.* 1997) with diagnosis
39 and treatment difficult to accomplish, exacerbated by a lack of basic biological information
40 coupled with high diversity in fishery cultures and structure (Li *et al.* 2002).

Fish farm infrastructure ranges from near natural systems to highly controlled artificial environments. Despite this diversity, all farms can experience temperature shifts due to climate change and/or increased use of technology (Jiang 2010). Temperature is crucial in farming, significantly influencing animal physiology and wellbeing. Associated parasites and disease are equally affected by temperature, which can lead to drastic shifts in infection dynamics. For parasites, high ambient temperatures typically lead to a short generation time as life history traits are completed more quickly; however, each trait can respond differently to temperature leading to trade-offs (e.g. Gophen 1976; Andersen and Buchmann 1998; Soleng *et al.* 1998; Sahoo *et al.* 2013). Examination of a suit of life history traits is therefore required to understand how temperature impacts overall parasite population dynamics. This is critical if we are to predict infection dynamics and develop more effective management practices.

One of the most conspicuous parasite groups plaguing fisheries are ectoparasitic fish lice, relatively large (compared to most fish parasites) crustaceans responsible for widespread damage in both marine and freshwater systems (Hakalahti *et al.* 2008; Costello 2009). Elevated temperatures are linked to outbreaks (Hakalahti *et al.* 2004a; Shimura 1983; Harrison *et al.* 2006), with modelling of marine sea lice showing a higher epidemic potential at higher temperatures (Groner *et al.* 2014). Freshwater lice (Genus *Argulus*), are particularly dependent on spring warming to induce hatching of overwintered eggs, which kick-start early population growth (Mikheev *et al.* 2001). As such, wild fisheries are predicted to encounter *Argulus* spp. earlier in the year and for prolonged periods under climate change scenarios, while in aquaculture systems maintained above 10 °C *Argulus* spp. can reproduce continuously (Hakalahti *et al.* 2006; Taylor *et al.* 2009a; Stewart *et al.* 2017). Freshwater lice are also a rising problem in UK angling fisheries; only one legal chemical treatment is currently available (Slice®, typically used against sea lice) with resistance a concern (Hakalahti *et al.* 2004b; Taylor *et al.* 2005). Management strategies focusing on stocking practices can help reduce infection depending on application (McPherson *et al.* 2012), while control methods such as egg-laying traps lack testing (Taylor *et al.* 2005). To improve current application of management, an understanding of how *Argulus* spp. dynamics in fisheries change under differing environmental conditions is needed.

Here, we examined the impact of temperature on one of the most common Eurasian freshwater fish lice, *Argulus foliaceus* (see Radkhah 2017)*,* before infection, during establishment and post-infection. Specifically, we aimed to identify changes in parasite hatching, growth and survival on and off the host. The impact of infection density and host species on parasite growth was also considered due to the variety of hosts across farming systems and tendency of *Argulus* to aggregate on the host. Temperature also has the potential to alter both of these factors by influencing host-parasite optima.

2. Methods

2.1 Host origins and maintenance

81 Three-spined sticklebacks (*Gasterosteus aculeatus*) were collected via hand netting from
82 Roath Brook, Cardiff (ST 18897 78541) on 19/04/18 and 20/06/18, with ornamental guppies
83 (*Poecilia reticulata*) purchased from a wholesaler on 29/06/18. Upon arrival at Cardiff
84 University, all fish were lightly anaesthetised with 0.02% MS222 (tricaine methanesulfonate)
85 and screened for ectoparasites using a dissection microscope with fibre optic illumination.
86 Both species were infected with *Gyrodactylus* spp.; guppies were treated with Levamisole
87 according to Schelkle *et al.* (2009) while for sticklebacks gyrodactylids were removed
88 manually with watchmaker's forceps due to the low prevalence. No *Argulus* spp. were found
89 on either fish species. All fish were acclimatised in a laboratory setting on a 12 h:12 h light:
90 dark cycle, fed daily and maintained in stock tanks at a density <1 fish/L for 2 weeks prior to
91 experimental use. Sticklebacks were maintained at 14 ± 0.5 °C and fed *Tubifex* bloodworm,
92 while guppies were kept at 24 ± 0.5 °C and fed Aquarian® tropical fish flakes. Prior to
93 experimental use, all fish were screened clear of ectoparasites three times (Schelkle *et al.*
94 2009) and measured for standard and fork length (using callipers accurate to 0.1 mm).
95 Throughout all experiments, location of *Argulus foliaceus* on the host was recorded to
96 examine parasite movement.

97   Permission was obtained from local authorities prior to fish collection. All animal work
98 was approved by the Cardiff University's Animal Ethics Committee, followed ARRIVE
99 guidelines and was conducted under Home Office Licence PPL 303424.

100

101 *2.2 Parasite cultures and infection*
102 *Argulus foliaceus* were obtained from laboratory cultures, maintained using three-spined
103 stickleback hosts (see Stewart *et al.* 2017). Two *A. foliaceus* strains were used in this study,
104 lab stain (cultured 4 years in lab, origins detailed in Stewart *et al.* 2017) and wild strain
105 (cultured 1 year in lab) originating from *A. foliaceus* adults (identified morphologically
106 according to Fryer 1982) obtained from Rudd (*Scardinius erythrophthalmus*) from a fishery in
107 Surrey on the 19th October 2017. To hatch *A. foliaceus* for experimental use, eggs laid in
108 culture were removed from storage at 7°C (development ceases <10 °C; Shafir and As 1986)
109 and gradually acclimated to incubation temperature (14 or 24 °C; experiment dependent) by
110 placing them at ambient air temperature to allow gradual warming of the water (10 h to reach
111 24 °C from 7 °C, 4 h to reach 14 °C). Temperatures were maintained using thermostatically
112 controlled rooms; average temperature = 24 °C ± 0.52 SD and 14 °C ± 0.55 SD. Eggs were
113 checked daily and given weekly water changes until hatching. No significant differences were
114 found between lab and wild strain *A. foliaceus* regarding incubation time, hatching
115 success/period and survival on/off the host (data not shown). As such, the most prolific
116 culture was used at the time of each experiment (lab strain for hatching and survival
117 experiments, wild strain for parasite growth experiments).

118   Infections were performed by placing a single fish into 100 ml water and introducing
119 the required number of parasites via a pipette. In all cases, *Argulus* naturally attached to the
120 fish within 10 min of exposure. For the hatching and survival experiments, time to infect was
121 recorded for each parasite, however, no significance was found between temperature

122 treatments (shock, gradual or no temperature change; $F_{3, 219}$ = 0.09, p = 0.96), with host length
123 ($F_{1, 218}$ = 0.01, p = 0.92) or with parasite position post infection ($F_{12, 206}$ = 0.66, p = 0.96). To
124 measure *A. foliaceus* on the host, infected fish were anesthetised using 0.02 % MS-222 and
125 placed under a dissecting microscope in a glass dish with 200 ml of dechlorinated water.
126 Images were taken of attached *A. foliaceus* with fish laying flat on their sides, at 10x
127 magnification using a Lumenera Infinity 1 camera with Infinity Capture software version 6.5.4.
128 *A. foliaceus* were measured from the rostral tip of their carapace to caudal end of the
129 abdominal lobes using ImageJ version 1.51j8 (Schneider *et al.* 2012). To measure *A. foliaceus*
130 off the host, parasites were placed onto a slide using a pipette, restrained by reducing their
131 pool of water to a minimal amount, and then imaged as above. All images were calibrated for
132 measurements using a 1/100 mm micrometre scale.

133

134 *2.3 Temperature impact on parasite hatching and survival*
135 To determine the effect of temperature on *A. foliaceus* hatching success and survival, three
136 temperature treatments were investigated: gradual temperature change (eggs incubated at
137 24 °C with newly hatched parasites gradually cooled to 14 °C over 24 h), shock temperature
138 change (eggs incubated at 24 °C with newly hatched parasites introduced to 14 °C water 24 h
139 post hatching without acclimation) and finally no temperature change (eggs incubated and
140 parasites maintained thereafter at 14 °C). For hatching success trials, three separate groups
141 of eggs were incubated per temperature (14°C N = 132 eggs total, 24°C N = 476 eggs) with
142 daily checks and weekly water changes.
143 For survival on the host, individual sticklebacks (average standard length = 40 mm ±
144 0.45) were infected with five individuals of *A. foliaceus* (all from the same temperature
145 treatment) 24 h post-hatching and placed into 1 L tanks at 14 °C (standard stickleback
146 infection level; Stewart *et al.* 2017, N = 15 fish and N = 75 parasites per treatment). Any *A.*
147 *foliaceus* lost during infection (N = 19 total) were presumed eaten and replaced (Bandilla *et*
148 *al.* 2008). *A. foliaceus* survival was monitored on infected sticklebacks daily for 7 days and
149 then weekly until 21 days post-infection.
150 Parasite survival off the host was assessed by placing newly hatched *A. foliaceus* into
151 50 ml dechlorinated water at 14 °C (N = 30-65 parasites per treatment). Here an additional
152 temperature treatment was tested with parasites hatched and maintained at 24 °C (N = 37
153 parasites). *A. foliaceus* were monitored daily using a dissecting microscope with the number
154 alive, moribund and dead recorded. Consistently, one day prior to death parasites were
155 moribund - as this displayed the same trend as survival, it is not reported further.

156

157 *2.4 Temperature, parasite density and host species impact on A. foliaceus growth*
158 To ascertain the impact of temperature on *A. foliaceus* growth, sticklebacks acclimatised to
159 14, 19 and 24 °C (1 week acclimation period) were infected with a single *A. foliaceus*
160 metanauplius measured prior to infection (day 0, length = 0.618 mm ± 0.049 SD; N = 15 fish
161 and parasites per temperature). To investigate any additional impact of host species and
162 infection density on *A. foliaceus* growth, sticklebacks and ornamental guppies were selected

163 as two extremes. Sticklebacks are a temperate, natural host found in most waterbodies across
164 the UK, versus guppies, a tropical fish and one of the most popular pet species with reports
165 of *A. foliaceus* infection in aquaculture/pet trade (Walker *et al.* 2007; Momeni Shahraki *et al.*
166 2014; Maceda-Veiga *et al.* 2016). For experimental work sticklebacks and guppies
167 acclimatised to 19 °C were infected with one individual of *A. foliaceus* per 7.4 mm of host
168 standard length (based on maximum non-lethal infection density of 5 parasites per
169 stickleback: Stewart *et al.* 2017; N = 10 fish per host species, N = 3 - 5 parasites per fish). Post-
170 infection, all fish were maintained individually in 1 L tanks with water changes every 48 h to
171 maintain water quality. One day post-infection (day 1) and subsequently every 48 h for two
172 weeks, *A. foliaceus* were measured on the host with their position noted. After 2 weeks, all *A.*
173 *foliaceus* were removed from fish, sexed and re-measured off the host to give final parasite
174 length.
175
176 *2.5 Statistical Analysis*
177 All statistical analyses were conducted in R statistical software v3.4.3 (R Core Team 2017)
178 using the following packages: "ggplot2" to visualise the data (Wickham 2009), "survival" to
179 run survival analyses (Therneau and Grambsch 2000; Therneau 2020) and "lme4" to run
180 Generalised Linear Mixed Models (Bates *et al.* 2014). Models were refined through stepwise
181 deletion of non-significant terms and Akaike information criterion comparisons, with visual
182 examination of model plots to check standardised residuals for normal distribution and
183 homogeneity of variance. In all mixed models fish ID was included as a random factor to
184 account for pseudo-replication, and in all tests the level of significance was taken as $p < 0.05$.
185   To examine the survival of *A. foliaceus* on stickleback hosts, a Generalised Linear
186 Mixed Model (GisedLMM) with Poisson family and square root link function was used with
187 number of days post infection, temperature treatment (gradual, shock or none), an
188 interaction between day and treatment and host standard length as dependent variables.
189 Survival analysis was used to determine the effect of temperature treatment and time on *A.*
190 *foliaceus* survival off the host. Hatching success of eggs was compared across treatments
191 using a Chi-squared test.
192   A GisedLMM with Gaussian family and log link function was used to assess the impact
193 of parasite sex, host standard length, host species, days post-infection, temperature and an
194 interaction between day and temperature on *A. foliaceus* length. To analyse the impact of
195 parasite density and host species on *A. foliaceus* length, two General Linear Mixed Models
196 (GLMM) were used to examine the effect of days post-infection and host standard length,
197 alongside either infection density and an interaction between infection density/day, or host
198 species with an interaction between host species/day.
199   Additionally, for the growth experiments, a GisedLMM with binomial family and logit
200 link function was used to assess whether parasite location (on the body of the host instead of
201 the fins, yes/no) was affected by temperature, infection density, host species and time. To
202 examine overall movement of *A. foliaceus* on hosts, a GisedLMM with binomial family and
203 logit link function compared whether a parasite moved (yes/no) to temperature, host species,

204   infection density, days post-infection and host length. Two-Proportion Z-Tests were also used
205   to compare the number of parasites on the body of the fish versus the fins across five parasite
206   size groups: 0.40 - 0.79, 0.80 - 1.19, 1.20 - 1.59, 1.60 - 1.99 and 2.00 - 2.39 mm. These size
207   ranges were based on *A. foliaceus* developmental stages (see Rushton-Mellor and Boxshall
208   1994), parasites larger than 2.4 mm length were not statistically assessed due to small sample
209   size.
210

211   **3. Results**
212   *3.1 Temperature impact on parasite hatching and survival*
213   At 24 °C, *Argulus foliaceus* eggs hatched after an average incubation period of 27 days (range
214   19 – 39 days) while at 14 °C eggs hatched after 67 days (range 60 – 75 days). Hatching success
215   of eggs across temperature treatment ranged from 57.7% to 63.7%, and success did not differ
216   between eggs incubated at 24 vs 14 °C ($\chi^2(1)$ = 2.36, p = 0.13).
217   *A. foliaceus* maintained at 24 °C off the host had significantly lower survival than
218   parasites maintained at 14 °C ($\chi^2(3)$ = 54.10, p < 0.001, survival analysis; figure 1). On
219   stickleback hosts, *A. foliaceus* survival still significantly decreased over time ($F_{1, 420}$ = 67.02, p
220   < 0.001, GLMM), with just under 50% survival 21 days post-infection (figure 1). Host length
221   did not significantly impact parasite survival ($F_{1, 417}$ = 2.63, p = 0.11, GisedLMM).
222   Survival of *A. foliaceus* on and off stickleback hosts was not impacted by incubation
223   temperature (24 or 14 °C; off host $\chi^2(1)$ = 1.60, p = 0.20, survival analysis, on host $F_{2, 418}$ = 0.48,
224   p = 0.78, GisedLMM) or temperature treatment post-hatching (gradual, shock or no thermal
225   change; off host $\chi^2(2)$ = 2.90, p = 0.23, survival analysis, on host $F_{1, 419}$ = 0.90, p = 0.52,
226   GisedLMM).
227

228   *3.2 Temperature, parasite density and host species impact on A. foliaceus growth*
229   *A. foliaceus* length increased with temperature and over time ($F_{2, 360}$ = 104.96, p < 0.001,
230   GisedLMM; figure 2). At 14 days post-infection, *A. foliaceus* length averaged 2.5 mm at 24 °C,
231   1.9 mm at 19 °C and 1.1 mm at 14 °C.
232   Parasite growth was significantly slower at high compared to low parasite density
233   (figure 2; $F_{1, 195}$ = 34.15, p < 0.001, GLMM). Parasite length was also affected by host species
234   over time ($F_{1, 156}$ = 11.69, p < 0.001, GLMM): when infected with multiple parasites, but at an
235   equivalent density, sticklebacks had larger *A. foliaceus* than guppies.
236   Considering *A. foliaceus* averaged 0.618 mm length at birth, adulthood (4.7 mm; taken
237   from Rushton-Mellor and Boxshall 1994; Taylor *et al.* 2009b) would take 124 days at 14 °C, 45
238   days at 19 °C and 30 days at 24 °C for low infection stickleback hosts (assuming a linear growth
239   pattern). For the higher infection density tests at 19°C, *A. foliaceus* would take 50 days to
240   reach adulthood on sticklebacks and 55 days on guppies. These values are however an
241   estimate as *Argulus* species do display diverse growth profiles (Rushton-Mellor and Boxshall
242   1994; Pasternak et al. 2004), especially under natural, wild conditions (Taylor et al. 2009b).
243   In all tests, host length and parasite sex did not significantly impact *A. foliaceus* growth
244   (host length; $F_{1, 362}$ = 1.31, p = 0.24, parasite sex; $F_{1, 363}$ = 3.14, p = 0.07, GLMM).

245

246   *3.3 Position and movement of A. foliaceus on hosts*

247   Significantly more *A. foliaceus* were found on the fins of hosts versus the body as temperature

248   and time spent on host increased ($F_{1, 822}$ = 4.60, *p* = 0.041 and $F_{1, 823}$ = 6.86, *p* = 0.008

249   respectively, GisedLMM). Parasite density and host species did not affect parasite position on

250   host ($F_{1, 822}$ = 0.01, *p* = 0.71 and $F_{1, 821}$ = 0.21, *p* = 0.65 respectively, GisedLMM). *A. foliaceus*

251   movement frequency was higher at high infection density and temperature ($F_{1, 446}$ = 50.80, *p*

252   < 0.001 and $F_{1, 445}$ = 24.89, *p* < 0.001 respectively, GisedLMM), but was not affected by host

253   species, time spent on host or host length ($F_{1, 444}$ = 0.27, *p* = 0.51, $F_{1, 446}$ = 1.43, *p* = 0.05 and $F_{1,}$

254    $_{442}$ = 0.20, *p* = 0.66 respectively, GisedLMM). *A. foliaceus* position was also significantly

255   influenced by parasite size; 70% of *A. foliaceus* 2.0 - 2.4 mm in length were located on the

256   host's body, versus 47% of newly hatched parasites 0.4 - 0.8 mm length ($\chi^2(1)$ = 4.36, *p* =

257   0.037, Two-Proportion Z-Test).

258

259   **4. Discussion**

260   Parasite generation time is intrinsically linked to environmental variables and here, *Argulus*

261   *foliaceus* responded positively to increasing temperature with faster incubation and growth.

262   However, life span off the host was reduced at higher temperatures, potentially impacting

263   infection success. *A. foliaceus* also demonstrated a high resistance to sudden temperature

264   changes, with a 10 °C temperature shock having no impact on parasite survival on or off the

265   host. Infection density and host species also affected parasite growth, variables which could

266   alter infection dynamic predictions especially as they can change drastically across

267   temperature and farming system.

268   Both egg incubation and *A. foliaceus* growth on hosts were significantly faster at 24 °C

269   compared to 14 °C, suggesting *A. foliaceus* at higher temperatures would experience a shorter

270   generation time as birth and development occur more rapidly. While fast life history traits

271   potentially allow parasites to rapidly exploit hosts, they may also limit infection depending on

272   host density; for example in entomopathogenic nematodes, prolific availability of hosts

273   benefits parasites with fast infection rates while limited host availability favours parasites

274   with slower rates (Crossan *et al.* 2007). Nematodes with fast infection rates also had a

275   corresponding trade off in fecundity and survival (Crossan *et al.* 2007), comparable to this

276   study where *A. foliaceus* at 24 °C experienced fast incubation and growth, but also a

277   significant reduction in survival off the host. High host densities (such as those encountered

278   in aquaculture systems) could override this trade off, negating any impact of reduced survival

279   at high temperatures. For angling fisheries however, replacement stocking to maintain low

280   fish densities has been previously predicted to decrease parasite populations (McPherson *et*

281   *al.* 2012). In this case parasite survival could potentially influence overall parasite population

282   success, as survival at 14 °C was double compared to 24 °C allowing parasites at low

283   temperatures more opportunities for infection. This low temperature survival was also

284   double the observed survival in a previous report by Walker *et al.* (2011a) examining 1 day

285   old *A. foliaceus* at 15 °C. This difference potentially arises from inter-population variation, the

286 1°C difference and/or the inclusion of aerators by Walker *et al.* (2011a), which could cause
287 higher parasite activity and subsequent increased metabolic cost/shorter lifespan.

288   *A. foliaceus* were maintained at constant temperature under laboratory conditions,
289 however in heterogenous environments the parasite could maximise survival by moving to
290 thermally optimal areas. *A. foliaceus* have a thermal preference of 28 – 30 °C (Herter 1927,
291 although shadows present in the experimental setup may have affected preference results;
292 Lagerspetz and Vainio 2006) suggesting they do not select cooler temperatures (such as 14 °C)
293 to increase off host survival. Regardless, the ability of any parasite to select preferential
294 microclimates should be considered when examining infections in fisheries and aquaculture.

295   Another key factor contributing to the success of *A. foliaceus* is their broad host range;
296 they are found on practically all fish species within their natural habitat, alongside successfully
297 infecting novel, unnatural hosts including ornamentals such as goldfish, koi and guppies
298 (Walker *et al.* 2007; Momeni Shahraki *et al.* 2014; Mirzaei and Khovand 2015). Despite a
299 difference in growth rate, *A. foliaceus* successfully infected and survived on both natural,
300 native stickleback hosts and novel guppy hosts. Possessing a broad host range allows parasites
301 to exploit a wider host pool, providing an advantage over specialist parasites in systems with
302 mixed host species (such as wild/angling fisheries). In comparison, monoculture aquaculture
303 systems will likely give rise to specialist parasites as they outcompete generalists. Regardless
304 of system, host availability should be considered when assessing problematic infections as
305 both specialist (Pasternak *et al.* 2004) and generalist (shown here) *Argulus* species show
306 differential growth across host species.

307   *Argulus* typically display an aggregated distribution within host populations (Bandilla
308 *et al.* 2005; Walker *et al.* 2008). Here higher *A. foliaceus* density on the host resulted in lower
309 parasite growth rate, as such parasite populations could display reduced growth as
310 aggregation increases. Regarding parasite position on host, parasites increasingly moved from
311 the host fins to the body as time progressed and at higher temperatures. *A. foliaceus* moved
312 to the body when they reached 2.0 - 2.4 mm length (7th developmental stage, at which point
313 the mouth-tube is fully developed; Rushton-Mellor and Boxshall 1994), indicating the start of
314 blood feeding. Juveniles feed only on mucous/skin cells, whereas the blood-feeding adults
315 cause greater host damage with the potential for secondary infections (Bower-Shore 1940;
316 Bandilla *et al.* 2006; Walker *et al.* 2011b). Prioritising identification and treatment of *A.*
317 *foliaceus* infections when parasites are <2 mm length (pre-blood feeding) would improve
318 control by addressing infections before they cause significant damage, as currently (in UK
319 angling fisheries) infections are primarily tackled once adults are present in high numbers.

320   Understanding the dynamics of parasites across their life cycle is critical for predicting
321 changes to infection. *Argulus* populations have been previously modelled to examine the
322 impact of control methods and predict population changes/generation time (Fenton *et al.*
323 2006; Taylor *et al.* 2009b; McPherson *et al.* 2012; Kumar *et al.* 2017). Of these studies, Taylor
324 *et al.* (2009b) used *A. foliaceus* length-frequency data to estimate egg incubation, hatching
325 period and maturation rate of different cohorts within an angling fishery. The authors
326 calculated a maturation time of 34 days at 24 °C, comparable to our estimate of 30 days with

the difference likely caused by variables such as host species and infection density (as shown here), and/or our use of a linear growth assumption for estimation. However, Taylor *et al.* (2009b) also observed an increase in hatching period with decreasing temperature (not observed here) suggesting that this trait may be governed by different seasonal variables such as light period (Bai 1981). Incorporating this new empirical data into existing and future models should therefore help verify outputs and improve modelling predictions. This in turn will help farmers select appropriate control methods and conditions to give the best trade-off for host growth and parasite restriction, reducing the impact of parasites on industry.

Competing interests

We declare that the authors have no competing interests. The views expressed here are those of the authors and not their parent organisation.

References

Andersen, P.S. and Buchmann, K. (1998). Temperature dependent population growth of *Gyrodactylus derjavini* on rainbow trout, *Oncorhynchus mykiss*. *J. Helminthol.* **72**(1):9–14. doi: https://doi.org/10.1017/S0022149X00000900.

Bai, A.S. (1981). Photic effects on embryonation and phototactic responses by the larvae of *Argulus siamensis*. *Proc. Indian Acad. Sci.* **90**(5):513–517. doi: https://doi.org/10.1007/BF03186029.

Bandilla, M., Hakalahti, T., Hudson, P.J. and Valtonen, E.T. (2005). Aggregation of *Argulus coregoni* (Crustacea: Branchiura) on rainbow trout (*Oncorhynchus mykiss*): a consequence of host susceptibility or exposure? *Parasitology* **130**(2):169–176. doi: https://doi.org/10.1017/S0031182004006407.

Bandilla, M., Valtonen, E.T., Suomalainen, L.-R., Aphalo, P.J. and Hakalahti, T. (2006). A link between ectoparasite infection and susceptibility to bacterial disease in rainbow trout. *Int. J. Parasitol.* **36**(9):987–991. doi: https://doi.org/10.1016/j.ijpara.2006.05.001.

367   Bandilla, M., Hakalahti-Sirén, T. and Valtonen, E.T. (2008). Patterns of host switching in the
368   fish ectoparasite *Argulus coregoni*. *Behav. Ecol. Sociobiol.* **62**(6):975–982. doi:
369   https://doi.org/10.1007/s00265-007-0523-y.

371   Bates, D., Mächler, M., Bolker, B. and Walker, S. (2014). Fitting Linear Mixed-Effects Models
372   using lme4. *J. Stat. Softw.* **67**(1):1–48. doi: https://doi.org/10.18637/jss.v067.i01.

374   Bower-Shore, C. (1940). An investigation of the common fish louse, *Argulus foliaceus* (Linn.).
375   *Parasitology* **32**(4):361–371. doi: https://doi.org/10.1017/S0031182000015869.

377   Burka, J.F., Hammell, K.L., Horsberg, T.E., Johnson, G.R., Rainnie, D.J. and Speare, D.J. (1997).
378   Drugs in salmonid aquaculture – A review. *J. Vet. Pharmacol. Ther.* **20**(5):333–349. doi:
379   https://doi.org/10.1046/j.1365-2885.1997.00094.x.

381   Costello, M.J. (2009). The global economic cost of sea lice to the salmonid farming industry. *J.*
382   *Fish Dis.* **32**(1):115–118. doi: https://doi.org/10.1111/j.1365-2761.2008.01011.x.

384   Crossan, J., Paterson, S. and Fenton, A. (2007). Host availability and the evolution of parasite
385   life-history strategies. *Evolution* **61**(3):675–684. doi: https://doi.org/10.1111/j.1558-
386   5646.2007.00057.x.

388   FAO. 2018. The state of world fisheries and aquaculture 2018 - Meeting the sustainable
389   development goals. Rome. Licence: CC BY-NC-SA 3.0 IGO.

391   Fenton, A., Hakalahti, T., Bandilla, M. and Valtonen, E.T. (2006). The impact of variable
392   hatching rates on parasite control: a model of an aquatic ectoparasite in a Finnish fish farm.
393   *J. Appl. Ecol.* **43**(4):660–668. doi: https://doi.org/10.1111/j.1365-2664.2006.01176.x.

395   Fryer, G. (1982). *The Parasitic Copepoda and Branchiura of British Freshwater Fishes: A*
396   *Handbook and Key*. Ambleside, Cumbria: Freshwater Biological Association. pp. 45–52

398   Gophen, M. (1976). Temperature effect on lifespan, metabolism, and development time of
399   *Mesocyclops leuckarti* (Claus). *Oecologia* **25**(3):271–277. doi:
400   https://doi.org/10.1007/BF00345104.

402   Granada, L., Sousa, N., Lopes, S. and Lemos, M.F.L. (2016). Is integrated multitrophic
403   aquaculture the solution to the sectors' major challenges? – a review. *Rev. Aquacult.*
404   **8**(3):283–300. doi: https://doi.org/10.1111/raq.12093.

406   Groner, M.L., Gettinby, G., Stormoen, M., Revie, C.W. and Cox, R. (2014). Modelling the
407   impact of temperature-induced life history plasticity and mate limitation on the epidemic

408 potential of a marine ectoparasite. *PLoS One* **9**(2):e88465. doi:
409 https://doi.org/10.1371/journal.pone.0088465.
410
411 Hakalahti, T., Karvonen, A. and Valtonen, E.T. (2006). Climate warming and disease risks in
412 temperate regions - *Argulus coregoni* and *Diplostomum spathaceum* as case studies. *J.*
413 *Helminthol.* **80**(2):93–98. doi: https://doi.org/10.1079/JOH2006351P.
414
415 Hakalahti, T., Pasternak, A.F. and Valtonen, E.T. (2004a). Seasonal dynamics of egg laying and
416 egg-laying strategy of the ectoparasite *Argulus coregoni* (Crustacea: Branchiura). *Parasitology*
417 **128**(6):655–660. doi: https://doi.org/10.1017/S0031182004004986.
418
419 Hakalahti, T., Lankinen, Y. and Valtonen, E.T. (2004b). Efficacy of emamectin benzoate in the
420 control of *Argulus coregoni* (Crustacea: Branchiura) on rainbow trout *Oncorhynchus mykiss*.
421 *Dis. Aquat. Organ.* **60**(3):197–204. doi: https://doi.org/10.3354/dao060197.
422
423 Hakalahti, T., Mikheev, V.N. and Valtonen, E.T. (2008). Control of freshwater fish louse *Argulus*
424 *coregoni*: a step towards an integrated management strategy. *Diseases of Aquatic Organisms*
425 **82**(1):67–77. doi: https://doi.org/10.3354/dao01971.
426
427 Harrison, A., Gault, N. and Dick, J. (2006). Seasonal and vertical patterns of egg-laying by the
428 freshwater fish louse *Argulus foliaceus* (Crustacea: Branchiura). *Dis. Aquat. Organ.* **68**:167–
429 173. doi: https://doi.org/10.3354/dao068167.
430
431 Herter, K. (1927). Reizphysiologische untersuchungen an der karpfenlaus (*Argulus foliaceus*
432 L.). *Z. Vgl. Physiol.* **5**(2):283–370. doi: https://doi.org/10.1007/BF00338018.
433
434 Jiang, S. (2010). Aquaculture, capture fisheries, and wild fish stocks. *Resour. Energy Econ.*
435 **32**(1):65–77. doi: https://doi.org/10.1016/j.reseneeco.2009.06.002.
436
437 Kumar, S., Sathish Kumar, T., Vidya, R. and Pandey, P.K. (2017). A prospective of
438 epidemiological intervention in investigation and management of argulosis in aquaculture.
439 *Aquac. Int.* **25**(1):303–325. doi: https://doi.org/10.1007/s10499-016-0030-0.
440
441 Lagerspetz, K.Y.H. and Vainio, L.A. (2006). Thermal behaviour of crustaceans. *Biol. Rev.*
442 **81**(2):237–258. doi: https://doi.org/10.1017/S1464793105006998.
443
444 Lewin, W.C., Arlinghaus, R. and Mehner, T. (2006). Documented and potential biological
445 impacts of recreational fishing: insights for management and conservation. *Rev. Fish sci.*
446 **14**(4):305–367. doi: https://doi.org/10.1080/10641260600886455.
447

448 Li, D., Fu, Z. and Duan, Y. (2002). Fish-Expert: a web-based expert system for fish disease
449 diagnosis. *Expert. Syst. Appl.* **23**(3):311–320. doi: https://doi.org/10.1016/S0957-
450 4174(02)00050-7.

452 Maceda-Veiga, A., Domínguez-Domínguez, O., Escribano-Alacid, J. and Lyons, J. (2016). The
453 aquarium hobby: can sinners become saints in freshwater fish conservation? *Fish Fish.*
454 **17**(3):860–874. doi: https://doi.org/10.1111/faf.12097.

456 McPherson, N.J., Norman, R.A., Hoyle, A.S., Bron, J.E. and Taylor, N.G.H. (2012). Stocking
457 methods and parasite-induced reductions in capture: Modelling *Argulus foliaceus* in trout
458 fisheries. *J. Theor. Biol.* **312**:22–33. doi: https://doi.org/10.1016/j.jtbi.2012.07.017.

460 Mikheev, V.N., Pasternak, A.F., Valtonen, E.T. and Lankinen, Y. (2001). Spatial distribution and
461 hatching of overwintered eggs of a fish ectoparasite, *Argulus coregoni* (Crustacea: Branchiura).
462 *Dis. Aquat. Organ.* **46**(2):123–128. doi: https://doi.org/10.3354/dao046123.

464 Mirzaei, M. and Khovand, H. (2015). Prevalence of *Argulus foliaceus* in ornamental fishes
465 [goldfish (*Carassius auratus*) and Koi (*Cyprinus carpio*)] in Kerman, southeast of Iran. *J. Parasit.*
466 *Dis.* **39**(4):780–782. doi: https://doi.org/10.1007/s12639-013-0406-2.

468 Momeni Shahraki, M., Asgari, M.R., Khamesipour, F. and Raissy, M. (2014). Prevalence *of*
469 *Argulus foliaceus* and fungal infections in some ornamental fishes [Discus (*Symphysodon*
470 *discus*), Dwarf Gourami (*Trichogaster lalius*) and Guppy (*Poecilia reticulata*)] in Isfahan City of
471 Iran. *Kafkas. Univ. Vet. Fak*. doi: https://doi.org/10.9775/kvfd.2014.10844.

473 Pasternak, A., Mikheev, V. and Valtonen, E.T. (2004). Growth and development of *Argulus*
474 *coregoni* (Crustacea: Branchiura) on salmonid and cyprinid hosts. *Dis. Aquat. Organ.* **58**(2–
475 3):203–207. doi: https://doi.org/10.3354/dao058203.

477 R Core Team (2017). *R: A language and environment for statistical computing.* R Foundation
478 for Statistical Computing, Vienna, Austria. URL: http://www.R-project.org/.

480 Radkhah, A.R. (2017). Introduction to some species of *Argulus* (Crustacea: Branchiura),
481 parasitic infections in the freshwater fishes. *JASEM.* **21**(7):1268-1271–1271. doi:
482 https://doi.org/10.4314/jasem.v21i7.7.

484 Rushton-Mellor, S.K. and Boxshall, G.A. (1994). The developmental sequence of *Argulus*
485 *foliaceus* (Crustacea: Branchiura). *J. Nat. Hist.* **28**(4):763–785. doi:
486 https://doi.org/10.1080/00222939400770391.
487

488   Sahoo, P.K., Mohanty, J., Hemaprasanth, Kar, B., Mohanty, B.R., Garnayak, S.K. and Jena, J.K.
489   (2013). Egg laying strategies and effect of temperature on egg development of *Argulus*
490   *siamensis*. *J Parasit Dis.* **37**(2):158–162. doi: https://doi.org/10.1007/s12639-012-0148-6.

492   Schelkle, B., Shinn, A.P., Peeler, E. and Cable, J. (2009). Treatment of gyrodactylid infections
493   in fish. *Dis. Aquat. Organ.* **86**(1):65–75. doi: https://doi.org/10.3354/dao02087.

495   Schneider, C.A., Rasband, W.S. and Eliceiri, K.W. (2012). NIH Image to ImageJ: 25 years of
496   image analysis. *Nat. Methods* **9**(7):671–675. doi: https://doi.org/10.1038/nmeth.2089.

498   Shafir, A. and As, J. G. (1986), Laying, development and hatching of eggs of the fish
499   ectoparasite *Argulus japonicus* (Crustacea: Branchiura). *J. Zool*, 210: 401-413.
500   doi:10.1111/j.1469-7998.1986.tb03645.x

502   Shimura, S. (1983). Seasonal occurrence, sex ratio and site preference of *Argulus coregoni*
503   Thorell (Crustacea: Branchiura) parasitic on cultured freshwater salmonids in Japan.
504   *Parasitology* **86**(3):537–552. doi: https://doi.org/10.1017/S0031182000050721.

506   Soleng, A., Jansen, P. and Bakke, T. (1998). Transmission of the monogenean *Gyrodactylus*
507   *salaris*. *Parasitol. Int.* **47**:319. doi: https://doi.org/10.1016/S1383-5769(98)80927-6.

509   Stewart, A., Jackson, J., Barber, I., Eizaguirre, C., Paterson, R., van West, P., Williams, C., Cable,
510   J. (2017). Hook, line and infection: a guide to culturing parasites, establishing infections and
511   assessing immune responses in the three-spined stickleback. In: Rollinson, D. and Stothard, J.
512   R. (eds.). *Adv. Parasit.* Academic Press, pp. 39–109. doi:
513   https://doi.org/10.1016/bs.apar.2017.07.001.

515   Taylor, N.G.H., Sommerville, C. and Wootten, R. (2005). *Argulus spp. Infections in UK Stillwater*
516   *Trout Fisheries*. Environment Agency: Science Report SC990019/SR2. pp. 1–57

518   Taylor, N.G.H., Wootten, R. and Sommerville, C. (2009a). The influence of risk factors on the
519   abundance, egg laying habits and impact of *Argulus foliaceus* in stillwater trout fisheries. *J.*
520   *Fish Dis.* **32**(6):509–519. doi: https://doi.org/10.1111/j.1365-2761.2009.01007.x.

522   Taylor, N.G.H., Wootten, R. and Sommerville, C. (2009b). Using length-frequency data to
523   elucidate the population dynamics of *Argulus foliaceus* (Crustacea: Branchiura). *Parasitology*
524   **136**(9):1023–1032. doi: https://doi.org/10.1017/S0031182009006520.

526   Therneau T (2020). A Package for Survival Analysis in R. R package version 3.1-12,
527   https://CRAN.R-project.org/package=survival.

528

529 Therneau T.M. and Grambsch P.M. (2000). *Modelling Survival Data: Extending the Cox Model*.
530 Springer, New York. ISBN 0-387-98784-3.

532 Walker, P., Harris, J., Van der Velde, G. and Wendelaar Bonga, S. (2007). Size matters:
533 stickleback size and infection with *Argulus foliaceus* (L., 1758) (Branchiura, Arguloida).
534 *Crustaceana* **80**:1397–1401. doi: https://doi.org/10.1163/156854007782605556.

536 Walker, P.D., Harris, J.E., van der Velde, G. and Bonga, S.E.W. (2008). Differential host
537 utilisation by different life history stages of the fish ectoparasite *Argulus foliaceus* (Crustacea:
538 Branchiura). *Folia Parasitol.* 55(2):141–149.

540 Walker, P.D., Russon, I.J., Duijf, R., Velde, D., Van, G. and Bonga, S.E.W. (2011a). The off-host
541 survival and viability of a native and non-native fish louse (*Argulus*, Crustacea: Branchiura).
542 *Curr. Zool.* **57**(6):828–835. doi: https://doi.org/10.1093/czoolo/57.6.828.

544 Walker, P.D., Russon, I.J., Haond, C., Van Der Velde, G. and Wendelaar-Bonga, S.E. (2011b).
545 Feeding in adult *Argulus japonicus* Thiele, 1900 (Maxillopoda, Branchiura), an ectoparasite on
546 fish. *Crustaceana* **84**(3):307–318.

548 Wedemeyer, G.A. (1997). Effects of rearing conditions on the health and physiological quality
549 of fish in intensive culture. In: Iwama, G.K., Pickering, A.D., Sumpter, J.P. and Schreck, C.B.
550 (Eds.), *Fish Stress and Health in Aquaculture*. Cambridge University Press. pp. 35–72.

552 Wickham, H. (2009). *Ggplot2: Elegant Graphics for Data Analysis*. Springer, New York, NY. pp.
553 1–201

555 Wood, E.M. (2001). *Collection of coral reef fish for aquaria: global trade, conservation issues*
556 *and management strategies.* Marine Conservation Society, UK. pp. 5–48

557
558

559 **Figure 1.** *Argulus foliaceus* survival off the host at 14 or 24 °C and on host (three-spined
560 stickleback; *Gasterosteus aculeatus*) at 14 °C. For on host survival, all fish began with five
561 metanauplii. Error bars represent 95% confidence intervals.
562
563 **Figure 2.** Impact of temperature, infection density and host species on *Argulus foliaceus*
564 growth. Three-spined sticklebacks (*Gasterosteus aculeatus*) were infected with one individual
565 *A. foliaceus* metanauplii at 14°C, 19 °C and 24 °C (infection density = low). Additional guppies
566 (*Poecilia reticulata*) and sticklebacks at 19 °C were infected with one *A. foliaceus* metanauplii
567 per 7.4 mm of host standard length (creating a starting infection number of 3-5 parasites per
568 fish, infection density = high). Error bars represent 95% confidence intervals.